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Chemical and Bioassay Analyses of Diesel and Biodiesel Particulate Matter: Pilot Study

FINAL REPORT

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for

Howard E. Haines
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The statements and conclusions in this report are those of the authors and not necessarily those of the University of California or the California Air Resources Board. The mention of commercial products, their source, or their use in connection with material reported herein is not to be construed as either actual or implied endorsement of such products.

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Abbreviations

REE	Rapeseed ethyl ester
PAH	Polycyclic Aromatic Hydrocarbon
LACMTA	Los Angeles Metropolitan Transit Authority
SIM	Selected Ion Monitoring
GC/MS	Gas Chromatography/Mass Spectrometer
USEPA	U.S. Environmental Protection Agency
DCM	Dichloromethane
MDL	Method Detection Level
RDL	Reliable Detection Level
RQL	Reliable Quantitation Level
QA	Quality Assurance
BDL	Below Detection Limit
BeP	Benzo(e)pyrene
BaP	Benzo(a)pyrene

SUMMARY

The exhaust from diesel fuel combustion is known to be a highly complex mixture of toxic compounds. Combustion products from fuel consisting of a mixture of diesel with rapeseed oil ethyl ester (REE) or from 100% REE also is a complex mixture of compounds. Any effort to determine the potential health effects of the emissions from these fuels would require extensive chemical and biological analyses. One approach to help evaluate potential human health effects from the mixture of compounds present in particulate matter is to use a short-term bioassay in conjunction with chemical analyses. Bioassays have been developed to measure a number of different health effects, including effects hypothesized to be at least in part responsible for chronic diseases. For example, some bioassays measure damage to genetic material, or DNA. This damage, referred to as genotoxic activity, is thought to be integral in the process of developing many types of cancer.

In collaboration with the University of Idaho, the Montana Department of Environmental Quality, and the U.S. Department of Energy, we investigated two important health-based components of diesel and biodiesel exhaust: 1) The concentrations of polycyclic aromatic hydrocarbons (PAHs - some suspected animal and human carcinogens and present in these emissions) and 2) The genotoxicity (DNA damaging capability) of the particulate extracts from these emissions.

Four different fuels were tested in a 1995 Dodge 3/4 ton pickup truck Cummins B (5.9 L, Turbo diesel): 1) 100% ethyl ester of rapeseed oil (REE) 2) 100% diesel 2-D low sulfur fuel 3) 20% REE + 80% diesel 4) 50% REE + 50%

diesel. Emissions from the truck were collected on filters under the controlled conditions of a chassis dynamometer-dilution tunnel facility at the Los Angeles County Metropolitan Transit Authority (LACMTA) facility. An EPA test cycle was followed throughout. The cycle incorporates two approximately equal sampling times (referred to as P1 and P2 parts of the cycle). Due to the limited amounts of samples, filters were cut in half to provide samples for chemical analyses and bioassay investigations.

For the chemical analyses, filter halves from the P1 and P2 filters were pooled and extracted. Deuterated PAH isotopes were added for quantitation of each PAH. The filter extracts were analyzed using a gas chromatograph/mass spectrometer (GC/MS) in the selective ion mode (SIM) which is a specific analyses for selected PAHs. The PAHs can be generally divided into two groups: 1) the semi-volatile PAHs (for example, phenanthrene - three connected benzene rings) and 2) the non-volatile PAHs (for example, benzo(a)pyrene - five connected benzene rings). In diesel emissions, the concentrations of these semi-volatile PAHs have been reported to be higher compared to the heavier non-volatile PAHs. We analyzed for both semi-volatile and non-volatile PAHs.

Use of 100% desel fuel without a catalytic converter and under the condition of a hot start resulted in the highest quantities of PAHs measured per mile. The exception was for benzo(a)pyrene and perylene which had higher total masses per mile with the 100% REE and 50% REE blend than with the 100% diesel fuel. Under the conditions of a cold start without catalyst, emissions of fluoranthene and benzo(ghi)perylene from100% REE were higher (µg / mile) than that from 100% diesel fuel, but pyrene was lower from the 100% REE fuel.

For the catalyst-equipped engine, PAHs such as phenanthrene, fluoranthene, and pyrene remained at an approximately equivalent emission rate (µg/mile) independent of the REE content in the fuel (ranging from 100% diesel to 100% REE). Further, in the catalyst-equipped engine, the more chemically reactive PAHs [for example, benzo(a)pyrene] were emitted at greater levels for the pure REE and some of the blended REE fuels than in emissions from 100% diesel fuel.

For the bioassay analyses, a simple modification of the Salmonella/microsome test (called the microsuspension assay) was used throughout. Each filter half from each part of the EPA cycle (P1 and P2) was tested individually for genotoxicity (the potential to damage DNA). Three doses of each filter extract were tested in duplicate. The slope of the linear portion of the dose-response curve was used to determine the specific activity or potency of each extract. The emissions of mutagenic compounds, expressed as revertant equivalents per mile, were determined from this potency value and the total mass of particulate matter collected.

For both the non-catalyst and catalyst-equipped engine, use of the 100% REE fuel produced in the lowest genotoxic (DNA-damaging) activity in the tests. Blended fuels in the non-catalyst-equipped engine produced less emissions than emissions than the 100% diesel fuel.

For the catalyst-equipped engine, the highest emissions were from the cold start 100% diesel fuel when compared to any of the hot start samples. The next highest to the cold start 100% diesel fuel was the 20% REE/diesel blend, followed by either the 50% REE/diesel blend or the hot start 100% diesel. The use of the 100% REE fuel resulted in the lowest emissions compared to the REE blends and 100% diesel fuels.

These pilot studies, differences in the total emission of genotoxic compounds from the catalyst-equipped engine compared to the non-catalyst-equipped engine are apparent. The catalyst-equipped engine in some cases had higher mass emissions (µg/mile) of certain PAHs.

These studies would benefit from a replication using larger sample size, and a trapping of the vapor-phase compounds in conjunction with the trapping and analyses of the particulate matter. The vapor-phase mutagenic compounds could then be compared to the particle phase and a more complete profile of emissions could be obtained. Further, the emissions with and without a catalyst need further investigation measuring both particle and vapor-phase. Finally, two procedural approaches are recommended for incorporation into the test plan: 1) tunnel blanks where a sampling of the tunnel without the engine running and conducted for identical times as the test cycle is recommended. 2) tunnel conditioning where filtered ambient air is drawn through the system for specified times prior to testing the next fuel is recommended to be incorporated into the test plan.

Introduction

Biodiesel fuel is a compression ignition fuel made from plant oils or animal fats. The fuel may be used neat or in blends with petroleum diesel. Before it can be used as a fuel, the vegetable oil is first chemically reacted with alcolhol by a process called transesterification. This chemical process produces an of the oil and glycerol. The glycerol is removed before the ester is used as a fuel. The esterified oil can be used by most diesel engines without modification of the engine. In general, biodiesel fuel is being promoted as a safer, cleaner burning, and biodegradable resource. The chemical characterization and toxicological test information of the emissions from biodiesel fuel combustion currently are under investigation. One group of toxicologically important compounds that may be present in the emissions are the polycyclic aromatic hydrocarbons, or PAH. Some PAHs are potent mutagens (cause DNA damage) and carcinogens in laboratory animals and in humans.

In collaboration with the University of Idaho, the Montana Department of Environmental Quality, and the U.S. Department of Energy, we investigated the concentrations of PAHs in the particulate matter of the exhaust collected from the diesel engine chassis dynamometer. We also investigated the genotoxicity (DNA damaging capability) of the particulate extracts. Four types of fuel were tested in the vehicle: 1) 100% ethyl ester of rapeseed oil (REE) 2) 100% 2-D diesel control fuel 3) 20% REE + 80% diesel 4) 50% REE + 50% diesel. Emissions were collected under controlled conditions of a chassis dynamometer-dilution tunnel facility at the Los Angeles County Metropolitan Transit Authority (LACMTA) facility.

Vehicle, Test Cycle, and Fuel Testing Matrix

Samples were from a 1995 Dodge 3/4 ton pickup truck equipped with a Cummins 5.9 liter turbocharged diesel engine. The 4x4 truck was rated at 8,600 lbs Gross Vehicular Weight and had 3,700 miles registered on the odometer. No engine modifications were made for any of the REE fuel combinations. Emission testing was conducted by the University of Idaho at the LACMTA)chassis dynamometer test facility. The EPA Heavy Duty Vehicle Cycle was used for all emission testing. This cycle duration is 1060 sec (Code of Fed Reg, 40, Part 86, Appendix 1, Cycle D) that consist of two approximately equal timed parts for sample collection designated P1 and P2. The fuel mixtures and the number of test cycles that were performed on the chassis dynamometer, both with and without a catalytic converter, are summarized in Table 1.

Table 1. Fuel mixtures tested.

Fuel	Start Type	No. Cycles
100% REE	Cold	1
100% REE	Hot	4
100% Diesel	Hot	3
100% Diesel	Cold	1
20% REE	Hot	3
50% REE	Hot	3

B. Preparation of Filter Samples

Filter samples were collected on precleaned 70 mm Teflon-coated glass fiber filters (Pallflex Fiberfilm T60A20). Each filter set consisted of a primary filter and a secondary filter. For each test cycle, separate filter samples collected representing the P1 and P2 portions of the cycle. Each filter was divided into approximately equal halves for chemical analyses and bioassay testing. The filters were divided by cutting the filters in half, weighing each half, and presenting a half filter for either chemical analysis or bioassay. Based on preliminary chemical analyses, we needed to combine the P1 and P2 half filters before extraction for the chemical analyses only due to limited amounts of sample necessary to quantitatively determine PAH concentrations. Based on preliminary tests for bioassay, we found that there was adequate amounts of sample to test P1 and P2 filters separately. A single filter was extracted and tested for the entire pilot study.

II. CHEMICAL ANALYSES

Materials and Methods

The extracts of biodiesel emission particulate matter were analyzed for18 PAHs. An isotope dilution method was used to improve PAH quantitation. Deuterated isotopes for most of the target PAHs were added to each sample extract prior to filter extraction to compensate for losses during sample preparation. The biodiesel filters were sonicated in dichloromethane (DCM), filtered, concentrated, and the samples were analyzed by GC/MS in the selective ion monitoring (SIM) mode.

Procedure for Particulate Analysis

The biodiesel filter samples collected from the LACMTA dynamometer facility were divided into two halves. One-half of each filter was used for bioassay analysis and the other half was used for PAH analysis. The front and the backup filter halves were also extracted together. To acquire adequate amounts of sample for analyses, the half filters from P1 and P2 runs were pooled, except for selected P1 and P2 filter halves that were tested individually during a preliminary study. Prior to extraction, the filter halves were placed into a pre-cleaned flask. To the flask was added 25 mls of dichloromethane (DCM), followed by adding 100 microliters of 14 deuterated PAHs from a solution with concentration of 600 pg/ul. Each filter sample was sonicated for 20 minutes. The extract was transferred to a holding flask. The extraction was repeated three additional times with 20 ml of DCM each time. All extracts were transferred to the holding flask. The entire extract was filtered through a Teflon filter (0.5 micron pore size) and

the extract concentrated to a final evaporative volume of 0.5 mls and further concentrated to 0.3 mls by a gentle stream of nitrogen.

The target analytes and the corresponding deuterated internal standards are listed in Table 2 along with the target and qualifier ions used to identify and quantitate the analytes. Retention times are also presented.

TABLE 2: Target Analytes and Internal Standards

COMPOUND	TARGET AND QUALIFIER IONS	RETENTION TIME (MIN)
Naphthalene-d_8	136, 68	13.03
Naphthalene	128, 129, 127	13.56
Acenaphthene-d_{10}	162,164,160	17.19
Acenaphthylene	152, 153, 151	16.83
Acenaphthene	153, 154, 152	17.26
Fluorene-d_{10}	176, 174, 177	18.44
Fluorene	166, 165, 167	18.50
Phenanthrene-d_{10}	188, 94, 90	20.71
Phenanthrene	178, 179, 177	20.76
Anthracene-d_{10}	188, 187, 97	20.83
Anthracene	178, 177, 179	20.89
Fluoranthene-d_{10}	212, 106	23.53
Fluoranthene	202, 203	23.57
Pyrene-d_{10}	212, 106	24.05
Pyrene	202, 200	24.09
Chrysene-d_{12}	240, 120, 236	26.98
Benz[a]Anthracene	228, 229, 227	26.97
Chrysene	228, 229, 227	27.04
Benzo[b]Fluoranthene-d_{12}	264, 132	20.91
Benzo[b]Fluoranthene	252, 253, 126	30.00
Benzo[k]Fluoranthene-d_{12}	264, 132	30.02
Benzo[k]Fluoranthene	252, 253, 126	30.09
Benzo[a]pyrene-d_{12}	264, 132	31.09
Benzo[e]Pyrene	252, 126	30.98
Benzo[a]Pyrene	252, 253, 126	31.18

TABLE 2: Target Analytes and Internal Standards (continued)

COMPOUNDS	TARGET ION AND QUALIFIER IONS	RETENTION TIME (MIN)
Perylene-d_{12}	264, 265, 260	31.43
Perylene	252, 126	31.52
Dibenz[ah]Anthracene-d_{14}	292, 293	35.27
Indeno[1,2,3-cd]Pyrene	276, 275, 138	35.21
Dibenz[ah]Anthracene	278, 279, 139	35.36
Benzo[g,h,i]Perylene-d_{12}	288, 144	35.83
Benzo[g,h,I]Perylene	276, 275, 138	35.89

Instrument and Instrument Conditions

A Hewlett Packard 5890 Series II gas chromatograph (GC) interfaced to a HP 5970A mass selective detector and equipped with a HP 8290 autosampler was used throughout for the chemical analyses. The GC was equipped with a 30 m x .25 mm ID J&W DB-5 (.25 micron film thickness) fused silica capillary column. Helium (99.999%) was used as the carrier gas. The GC was run in a splitless mode with electronic pressure pulse programing. Following the pressure pulse program, the GC was run in both temperature program and constant pressure mode with vacuum compensation. The MSD was run in selective ion monitoring (SIM) or electron impact modes.

Calibration

The mass spectrometer was manually tuned using perfluorotributylamine prior to analyzing each set of samples. The mass spectrometer was optimized for SIM analysis of PAHs. A sample blank was injected into the GC to determine if any background contamination was present. This background information was followed by developing a calibration curve using five concentrations of each of the PAHs. The curve is used to quantitate the concentrations of PAHs in the filter extracts. The internal standards used in the chemical analyses are listed in Table 2. Filter extracts were injected after analysis of the calibration standards. A calibration check sample was conducted after every 10th sample to ensure that the instrument was properly calibrated.

Chemicals

Dichloromethane (OmniSolve, EM Science) was used throughout to preclean glassware and to extract filter samples. Naphthalene-d_8, acenaphthene-d_8, phenanthrene-d_{10}, chrysene-d_{10}, perylene-d_{12} were from Accustandard. All other deuterated standards were from Cambridge Isotopes Laboratories. Benzo[e]pyrene and perylene were from Chemical Services.

Detection Limit

A modified version of the proposed detection limits definitions as defined by the U.S. Environmental Protection Agency and the American Chemical Society (EPA/ACS) was used to report low level data. The method detection level (MDL) is defined as the Student's T-test multiplied by the standard deviation of 7 replicate analyses of a low level standard spiked in the sample matrix. The MDL is the lowest level at which an analyte can be reliably detected. The reliable detection level (RDL) is the lowest level at

which an analyte not detected is reliable. This value is two times the MDL. The reliable quantitation level (RQL) is the lowest level at whcih an analyte can be quantitated and is four times the MDL. The EPA/ACS detection level requires that the detection limit be determined in the actual sample matrix. Since the biodiesel matrix contained varying levels of all PAHs, the detection limits were based on a reagent spike. The MDL, RDL, and RQL for the PAHs of interest are presented in Table 3.

Table 3: Method Detection, Reliable Detection, and Reliable Quantitation Levels.

COMPOUND	MDL (pg/ul)	RDL (pg/ul)	RQL (pg/ul)
Naphthalene	2	3	6.15
Acenaphthylene	3	6	12.7
Acenaphthene	2	4	8.58
Fluorene	2	8	15.2
Phenanthrene	3	7	13.7
Anthracene	6	12	24.8
Fluoranthene	1	2	4.85
Pyrene	1	2	3.45
Benzo[a]anthracene	4	8	16.0
Chrysene/Triphenylene	2	5	9.91
Benzo[b]flouranthene	3	5	10.9
Benzo[k]flouranthene	3	5	10.9
Benzo[e]pyrene	1	1	2.76
Benzo[a]pyrene	2	4	9.00
Perylene	2	4	8.10
Indeno[1,2,3-cd]pyrene	4	8	16.0
dibenz[ah]anthracene	2	4	8.03
benzo[g,h,I]perylene	2	5	9.31

<MDL: Values below the method detection level.
>MDL-<RQL: Values between the method detection level and the reliable quantitation level.
RDL: Reliable Detection Level.

RESULTS

Particulate samples were collected from a diesel engine using 100% REE, blends of REE with diesel fuel, and 100% diesel fuel as described by Peterson and Reece (1995). The engine was equipped at different times with or without a catalyst samples were collected from both cold and hot start cycle samples. A filter sample from each test condition was analyzed for 18 different PAHs. Preliminary PAH analyses to determine the levels of PAHs present were performed on P1 filter samples 1430, 1433, and 1443. The filters were selected REE samples where little was known about the potential PAH content of the samples. These results revealed that the sample extracts required further concentration and use of a combined P1 and P2 filter samples, rather than the single P1 or P2 sample to obtain measurable concentrations for all PAHs except phenanthrene, pyrene, and fluoranthene. Phenanthrene, pyrene, and fluoranthene were present in measureable levels with a single filter half. However, the other PAHs were near or below levels of method detection. Therefore, for all subsequent filter samples, the P1 and P2 portions were extracted together and the extract chemically analyzed. This pooling of samples still allowed us to report PAH concentrations for each entire cycle.

Since PAH mass was measured on one-half filter, the total PAH mass collected on the whole filter had to be determined by calculation. This was accomplished by taking the mass of PAH present on the half filter and dividing it by the particulate mass on this half filter, resulting in µg of PAH per µg of particulate matter. This value was then multiplied by the particle mass of the filter which resulted in the PAH mass per filter, as summarized in Table A3-1.

Total PAH Per Filter

For the hot start samples without a catalyst, the highest emissions for the semi-volatile compounds such as phenanthrene are from the 100% diesel fuel. There appears to be little difference between the diesel-REE blends and the 100% REE. Cold start samples were only collected for the 100% diesel and 100% REE, and the total amounts of phenanthrene, pyrene, and chrysene/triphenylene are higher for the 100% diesel than for the 100% REE. For the 100% REE (cold start), fluoranthene, B[e]P, Benzo(a)pyrene and benzo(ghi)perylene were higher than for the 100% diesel.

For the hot start samples acquired from the vehicle equipped with a catalyst, many of the semi-volatile PAHs, such as fluorene were below the method detection level. Higher molecular weight PAHs such as benzo(a)pyrene, were present in higher amounts in the 100% REE and 50% REE than in the 20% REE and 100% diesel samples. For the cold start samples, phenanthrene was considerably higher in the 100% diesel compared to the 100% REE, while benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, and benzo(ghi)perylene, were present in higher total mass per filter in the 100% REE compared to the 100% diesel.

Mass of PAHs per Mass of Particulate Matter

Mass concentrations based on a per mass of particulate matter (specific mass concentrations) were calculated. Specific mass concentrations based on mass of PAH per mass of particulate matter (ng/g) were obtained by dividing the total PAH per filter by the total particulate mass per filter. Pooling the P1 with the P2 portion of the cycles precluded any comparisons between these portions. The results are presented in Table A3-2. For hot start samples acquired from the catalyst-equipped vehicle, the semivolatile PAHs such as phenanthrene and pyrene were present in

approximately equivalent concentrations independent of fuel type. The higher molecular weight PAHs such as benzo(a) pyrene, were present at the highest concentrations when using the 100% REE fuel. For the cold start samples, phenanthrene was present in higher concentrations in the 100% diesel fuel samples, while benzo(a)pyrene and benzo(ghi)perylene were present in higher concentrations in the 100% REE fuel.

For hot start samples acquired from the vehicle without catalyst , the semi-volatile PAHs such as phenanthrene were present in approximately equivalent concentrations independent of fuel type. The concentration of phenanthrene was lower overall compared to the concentrations detected in the samples from the catalyst-equipped vehicle. For the cold start samples using 100% REE and 100% diesel fuel, phenanthrene and pyrene were present in higher concentrations in the 100% diesel fuel samples. Fluoranthene was present in higher concentrations in the 100% REE fuel samples.

PAH Emissions

Diesel PAH emissions are reported on a microgram per mile basis (µg/mi). Results for the catalyst-equipped and the non-catalyst engine tests are reported in Table 4 and 5, respectively. For hot start samples from the catalyst equipped vehicle, the semi-volatile PAHs had emissions that were approximately equivalent and independent of fuel type. The higher molecular weight PAHs such as benzo(a)pyrene were present in higher concentrations in the 100% REE fuel emission samples. For the cold start samples from the catalyst-equipped vehicle, higher emissions (µg / mile) of phenanthrene were emitted in the 100% diesel fuel compared to the 100% REE fuel samples. More benzo(a)pyrene and benzo(ghi)perylene were emitted from the combustion of 100% REE fuel.

For the hot start samples from the vehicle without a catalyst, the 100% diesel fuel emitted higher quantities of phenanthrene, fluoranthene and pyrene compared to the 100% REE and the other blends. For the cold start samples from the vehicle without a catalyst, the emission of pyrene appeared to be higher from the 100% diesel fuel than from the 100% REE fuel samples. The emission of fluoranthene appeared higher for the 100% REE afuel.

TABLE 4. PAH EMISSIONS FROM A CATALYST-EQUIPPED DIESEL VEHICLE (μg/mile)

Sample ID	1430	1437	1433	1443	1440	1436
Filter ID	P1P2	P1,P2	P2	P2	P1,P2	P1
Percent Diesel	0%	100%	0%	50%	80%	100%
Catalyst	Yes	Yes	Yes	Yes	Yes	Yes
Hot/Cold Start	Cold	Cold	Hot	Hot	Hot	Hot
Total Particulates (g/mile)	0.1733	0.2358	0.1189	0.145	0.1328	0.1062
			PAH EMISSIONS (μg/mile)			
COMPOUND						
Naphthalene	3.40	2.55	15.19	6.19	2.08	7.00
Acenaphthylene	1.66	<.321	1.50	<.694	<.321	<.695
Acenaphthene	1.71	<.216	1.68	<1.68	<.216	<.470
Fluorene	.866	<.309	.526	<.670	<.309	<.672
Phenanthrene	5.47	27.66	6.65	13.99	9.41	9.62
Anthracene	2.12	1.91	1.78	<1.353	<.628	<1.36
Fluoranthene	9.51	8.94	9.07	8.81	6.86	6.87
Pyrene	12.04	14.98	8.82	11.52	10.63	10.37
Benz[a]anthracene	2.81	1.46	2.15	<.873	<.405	<.876
Chrysene/Triphenylene	3.42	2.30	2.70	1.42	1.91	<.542
Benzo[b]fluoranthene	3.41	1.33	2.16	.617	0.71	<.597
Benzo[k]fluoranthene	2.56	<.276	1.93	<.595	<.155	<.597
Benzo[e]pyrene	1.09	0.73	0.50	.366	<.0700	<.151
Benzo[a]pyrene	3.02	0.79	1.78	<.491	<.228	<.492
Perylene	<.204	<.205	<.445	<.442	<.205	<.444
Indeno[1,2,3-dc]pyrene	3.32	0.85	1.84	<.873	<.405	<.876
Dibenz[ah]anthracene	2.15	<.236	1.85	<.428	<.203	<.439
Benzo[ghi]perylene	4.53	1.18	2.13	<.508	<.235	<.509

ug/mile = micrograms per mile

< = less than the method detection level

TABLE 5. PAH EMISSIONS FOR NON-CATALYST DIESEL VEHICLE. (μg/mile)

Sample ID	1445	1454	1448	1458	1451	1456
Filter ID	P1,P2	P1,P2	P1,P2	P1,P2	P1,P2	P1,P2
Percent Diesel	0%	100%	0%	50%	80 %	100%
Catalyst	No	No	No	No	No	No
Hot/Cold Start	Cold	Cold	Hot	Hot	Hot	Hot
Emission Rate (g/mile)	0.4734	0.3557	0.2594	0.2484	0.192	0.6427
			PAH EMISSIONS (μg/mile)			
COMPOUND						
Naphthalene	2.30	2.17	2.20	2.65	1.44	7.31
Acenaphthylene	<3.18	<.320	<.316	<.320	<.319	<1.11
Acenaphthene	<.214	<.216	<.215	<.216	<.215	<.476
Fluorene	<.305	<.308	<.306	<.308	<.305	<1.06
Phenanthrene	27.54	37.90	10.20	11.11	10.28	35.68
Anthracene	<.620	2.63	<.620	<.623	<.620	<2.16
Fluoranthene	28.98	13.49	6.05	5.24	5.39	14.18
Pyrene	16.94	29.63	9.24	9.29	10.84	37.68
Benz[a]anthracene	2.31	2.98	.933	1.15	1.17	3.15
Chrysene/Triphenylene	2.57	4.24	1.17	1.49	1.55	4.77
Benzo[b]fluoranthene	2.73	2.51	1.10	1.33	1.00	2.54
Benzo[k]fluoranthene	2.09	.719	<.272	<.276	<.273	<.951
Benzo[e]pyrene	1.39	1.26	.642	.725	.551	1.71
Benzo[a]pyrene	1.38	1.00	.547	.519	<.225	<.752
Perylene	0.70	<.205	.452	.424	<.204	<.681
Indeno[1,2,3-dc]pyrene	1.61	1.15	<.399	<.402	<.401	<1.34
Dibenz[ah]anthracene	<.201	<.203	<.201	<.202	<.202	<.675
Benzo[ghi]perylene	2.85	1.53	.905	.912	.636	1.83

ug/mile = micrograms of PAH per mile

< = less than the method detection level

To ensure the validity of the data, only PAH levels above the RQL were reported in Tables 4, 5, A3-1, and A3-2. PAHs detected, but below the RQL were not included in these tables. Approximately one-fourth of the PAHs were detected but not quantified because they were between the MDL and the RQL. The PAHs reported here are from one-half filter for the P1 part of the cycle and one-half filter for the P2 part of the cycle. Detection is dependent on the amount of sample available for extraction.

The PAH emission rates (microgram per mile) are illustrated in Figures 1 through 9. Only the PAHs with the highest emission rates are shown. Each figure depicts the entire fuel test matrix for each PAH which consisted of collecting emissions from non-catalyst and catalyst-equipped diesel vehicle tests, as well as collecting the particulate matter during both cold and hot cycles.

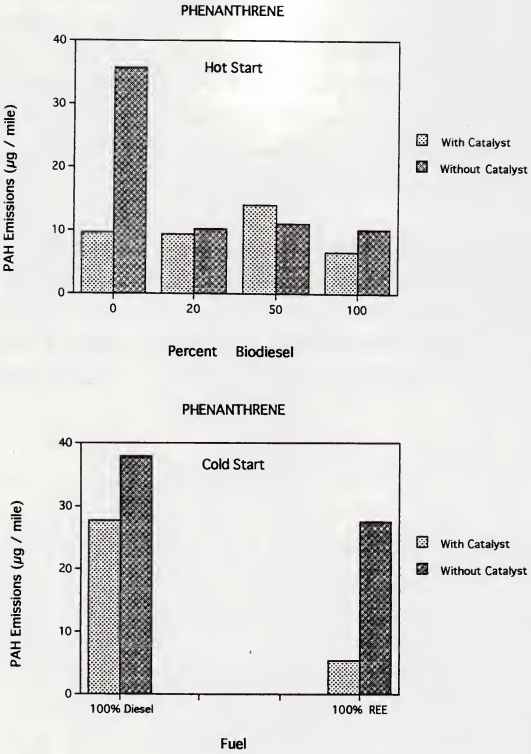


Figure 1. Phenanthrene emissions from a diesel engine equipped with and without catalyst. Upper panel represents the hot start data. Lower panel represents the cold start results for the 100 % diesel or 100% REE fuels.

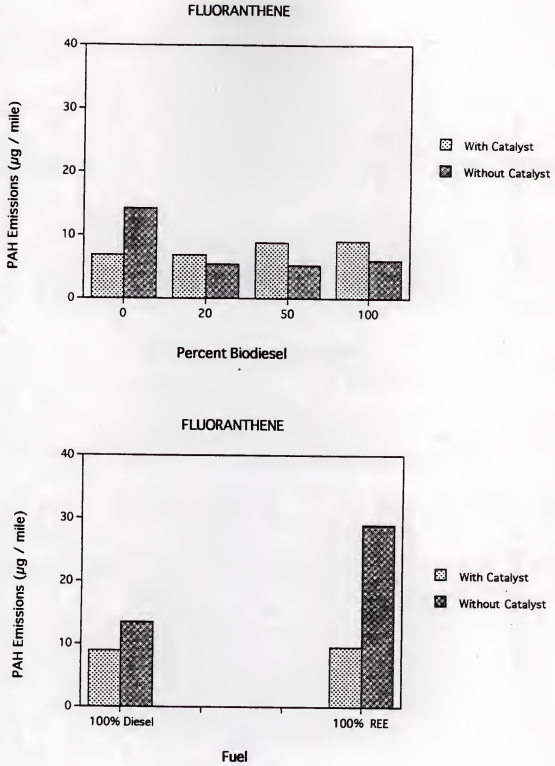


Figure 2. Fluoranthene emissions from a diesel engine equipped with and without catalyst. Upper panel represents the hot start data. Lower panel represents the cold start results for the 100 % diesel or 100% REE fuels.

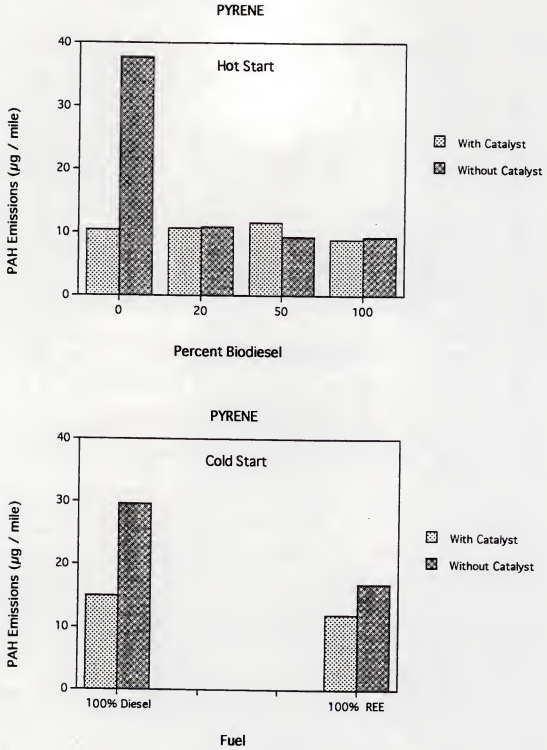


Figure 3. Pyrene emissions from a diesel engine equipped with and without catalyst. Upper panel represents the hot start data. Lower panel represents the cold start results for the 100 % diesel or 100% REE fuels.

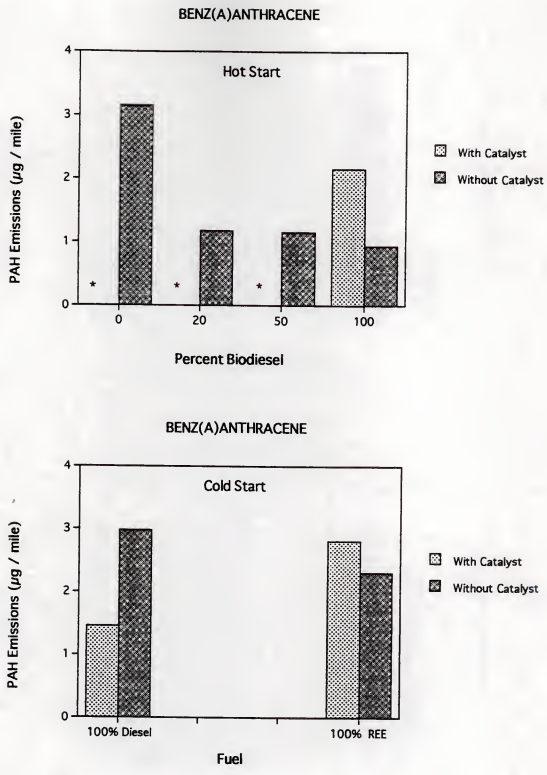
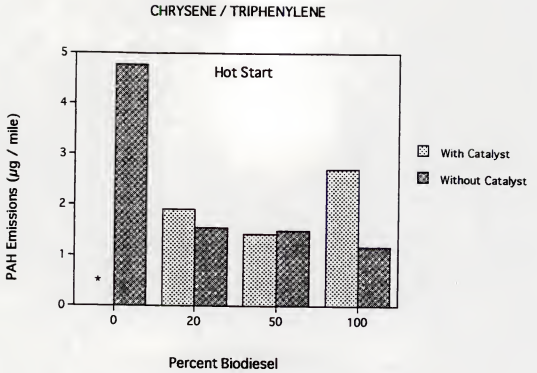


Figure 4. Benz(a)anthracene emissions from a diesel engine equipped with and without catalyst. Upper panel represents the hot start data. Lower panel represents the cold start results for the 100 % diesel or 100% REE fuels. (*) = data below the reliable quantitation level.



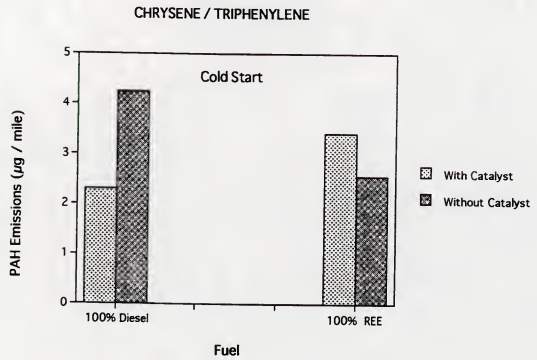


Figure 5. Chrysene/Triphenylene emissions from a diesel engine equipped with and without catalyst. Upper panel represents the hot start data. Lower panel represents the cold start results for the 100 % diesel or 100% REE fuels. (*) = data below the reliable quantitation level.

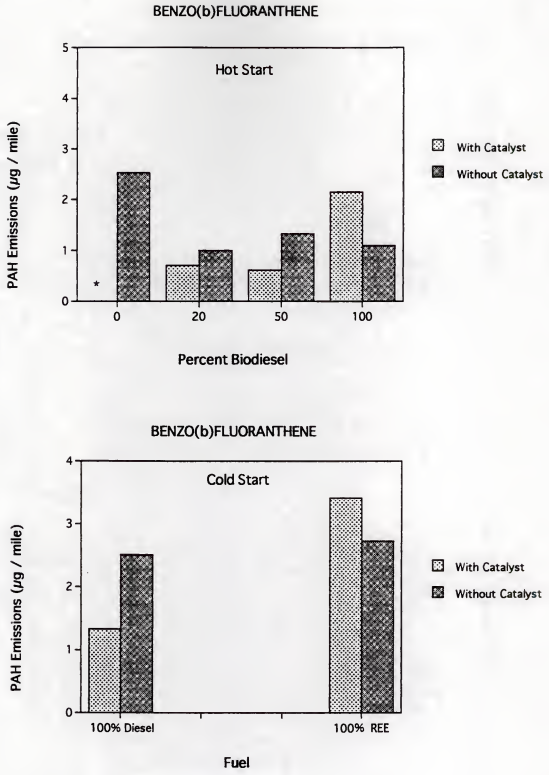


Figure 6. Benzo(b)fluoranthene emissions from a diesel engine equipped with and without catalyst. Upper panel represents the hot start data. Lower panel represents the cold start results for the 100 % diesel or 100% REE fuels. (*) = data below the reliable quantitation level.

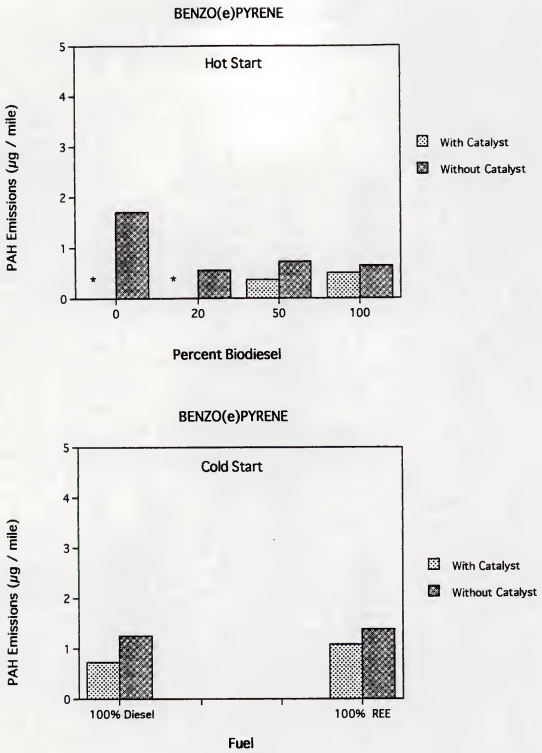


Figure 7. Benz(e)pyrene emissions from a diesel engine equipped with and without catalyst. Upper panel represents the hot start data. Lower panel represents the cold start results for the 100 % diesel or 100% REE fuels. (*) = data below the reliable quantitation level.

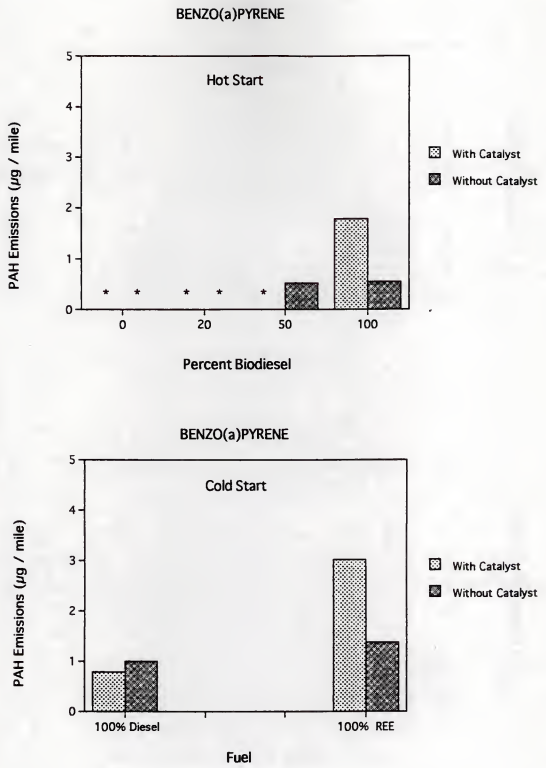


Figure 8. Benz(a)pyrene emissions from a diesel engine equipped with and without catalyst. Upper panel represents the hot start data. Lower panel represents the cold start results for the 100 % diesel or 100% REE fuels. (*) = data below the reliable quantitation level.

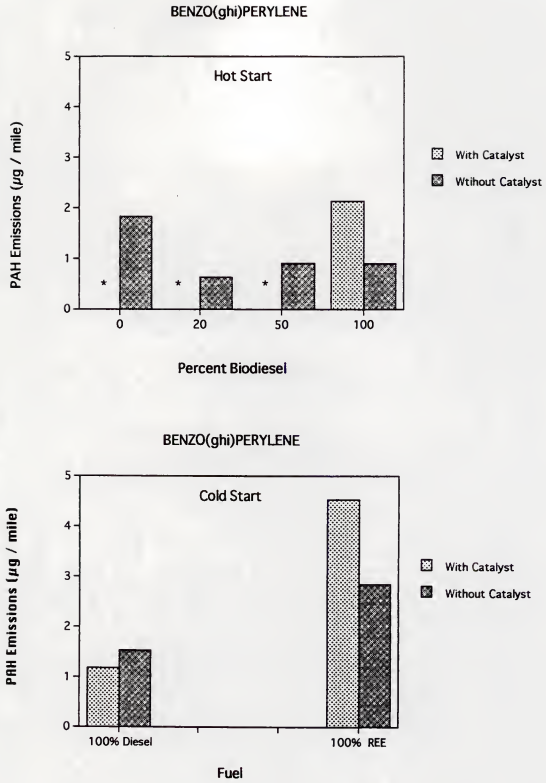


Figure 9. Benzo[ghi]perylene emissions from a diesel engine equipped with and without catalyst. Upper panel represents the hot start data. Lower panel represents the cold start results for the 100 % diesel or 100% REE fuels. (*) = data below the reliable quantitation level.

DISCUSSION

The more volatile PAHs such as phenanthrene, in general, are not efficiently trapped on the filter used to collect particulate matter. The amounts of these PAHs are therefore less than the amounts emitted. As the vapor pressure of a PAH increases, it becomes more volatile and is trapped less efficiently on the filter. However, using a conservative methodology for chemical analyses, the more volatile PAHs were detected and quantitated in our filter samples. The inefficient trapping of some of the more volatile PAHs would be most noticeable with naphthalene which contains 2 benzene rings, but efficently would be greater for PAHs containing 5 or more rings. Also, the concentration of naphthalene in the blank was approximately 40 picograms per microliter (pg/ul) of extract. Most of the sample concentrations were at similar levels and were therefore considered to be at the background concentration.

For the 100% 2-D diesel fuel, the amount of PAHs emitted by the engine equipped with a catalytic converter was significantly lower than in the non-catalyst equipped engine. This was observed in both the cold and hot starts. Generally, for the REE mixtures, the non-catalyst engine emitted less PAHs than the same engine running on 100% diesel fuel. Benzo(a)pyrene was an exception, however, with more BaP produced by using either the 100% REE or the 50% REE fuel than by 100% 2-D diesel fuel. In general, PAH emissions from the 100% REE fuel and REE/diesel blends remained relatively equivalent (in the non-catalyst engine). Benzo(a)pyrene was an exception and increased in concentration as the percentage of REE increased.

In the engine equipped with a catalytic converter, and under hot start conditions, the semi-volatile PAHs such as phenanthrene, fluoranthene, and pyrene remained at relatively equivalent levels, regardless of the percentage of REE in the fuel. The engine equipped with a catalytic converter was less efficient at reducing the amounts of some of the more volatile PAHs such as phenanthrene, flouranthene, and pyrene. However, the catalytic converter was more efficient than the non-catalyst equipped engine at removing the less volatile PAHs such as benz[a]anthracene, benzo(e)pyrene, benzo(a)pyrene, and benzo[ghi]perylene. In the catalyst equipped engine, the efficiency of PAH removal appears to decrease as the percentage of REE fuel increases. For the 20 % REE and 50 % REE fuels, concentrations of these less volatile PAHs are below their detection limit. For the 100 % REE fuel, the levels increase beyond what was detected in the non-catalyst equipped engine. With 100% REE fuel, the PAH emissions with the catalyst become as high or higher than without the catalyst.

There are a number of factors that may be responsible for the observed differences between the catalyst and non-catalyst-equipped engine, especially with respect to increases in certain PAHs with the catalyst. One factor may be an inherent rate of chemical conversion or alteration of the PAHs by the catalyst. The catalyst was apparently designed to run with diesel fuel and to control particulate matter. One possibility of our observed results could be a difference in PAH chemical reactivity. Nielson (1993) has characterized chemical reaction rates of various PAHs and has classified these rates into 5 different categories. The most reactive PAHs such as pentacene are in class 1, while the most stable PAHs such as phenanthrene and fluoranthene are in class 5. Using the Nielson reactivity scheme, the least reactive PAHs tend to have modest changes in emissions with

increasing percentage of REE fuel, while the emission profile of the most reactive PAHs changes with increasing percentage of REE fuel. Perylene for example, is considered to be a class 2 reactive compound and is quantifiable only when using the 100% REE fuel.

When using the 100% REE fuel, the catalyst appears to inefficiently catalyze the chemical conversion or eliminate the class 2 reactive PAHs such as perylene. In 100% REE fuel, these reactive PAHs appear at concentrations as high or higher than in the non-catalyst equipped engine. At lower percentages of REE fuel, the catalyst appears to be more efficient where even the less reactive PAHs from the combustion of diesel fuel are converted to other compounds.

For the 100% REE fuel under both hot and cold starts, benzo[a]anthracene, benzo[b]fluoranthene, Benzo(a)pyrene, chrysene, and benzo[ghi]perylene emissions from the catalyst-equipped engine were higher than those from the non-catalytic system. This suggests that the REE fuel may affect the performance of either the engine or the catalyst. Another possibility is that the sequence of testing at the chassis dynamometer facility. For example, a cycle run using 2-D diesel fuel followed by a 100% REE run could affect the results of the REE fuel. Incorporation of blank tunnel runs prior to selected fuels would help evaluate potential cross contamination effects.

These results require further confirmation and should not be extrapolated to other engines, test conditions, or types of biodiesel fuels. Additional studies might include testing other types of catalysts and engines.

BIOASSAY

Introduction

The exhaust from diesel fuel combustion is known to be a highly complex mixture of compounds, including polycyclic aromatic hydrocarbons (PAHs) and their derivatives. The combustion products from the mixture of diesel with REE or from 100% REE are also considered to comprise a complex mixture of compounds. One approach to help chemically analyze the mixture of compounds present in particulate matter, and to evaluate its potential public health effects, is to use bioassay. Bioassays have been developed to measure a number of different health effects, including effects hypothesized to be at least in part responsible for chronic diseases. For example, there are bioassays that measure damage to genetic material (DNA). This damage, or genotoxic activity, is thought to be an important part of the process of developing cancer.

For chemical analyses, the bioassay typically serves as a chemical detector with a biological endpoint of genotoxicity (toxicity to DNA). This approach has been successfully used to chemically characterize diesel particulate matter, for example. The genotoxic activity also serves as an index of DNA damage and provides some indication as to the potency of the extract.

The exhaust from diesel engines has been determined to be a probable carcinogen to humans, and whole diesel exhaust was carcinogenic in a number of animal studies (IARC, 1989). Chemical extracts of particulate matter from diesel exhaust contain many genotoxic compounds, including many that are carcinogenic. The bioassay approach can be used to compare the

genotoxicity of particles from biodiesel to that produced from the compustion of diesel fuel.

The most widely used and validated short-term test for genotoxicity is the Salmonella/microsome test (Ames et al., 1975). The assay has been used for the screening of potentially carcinogenic compounds and in mechanistic toxicologic studies. The bioassay we routinely use is a microsuspension procedure previously developed and reported by our lab (Kado et al., 1983, 1986), which is a simple modification of the *Salmonella*/microsome test (Ames et al., 1975). The modified assay is approximately 10 times more sensitive than the original Ames*Salmonella* microsome procedure, based on absolute amounts of material added per tube. This test produces a reading of the mutagenic activity per microgram of particulate matter and mutagenic activity per mile.

Materials and Methods

A microsuspension procedure previously reported by Kado et al. (1983, 1986), which is a simple modification of the *Salmonella*/microsome test of Ames et al. (1975), was used throughout.

Tester strain TA98 was kindly provided by Dr. B.N. Ames, Berkeley, CA. For the microsuspension procedure, bacteria were grown overnight in Oxoid Nutrient Broth No. 2 (Oxoid Ltd., Hants, England) to approximately 1 - 2 x 10^9 cells/ml and harvested by centrifugation (5,000 x g, 4°C, 10 min). Cells were resuspended in ice-cold phosphate-buffered saline (0.15M PBS, pH 7.4) to a concentration of approximately 1 x 10^{10} cells/ml.

The S9 (metabolic enzymes) and S9 mix (enzyme co-factors) were prepared according to the procedure of Ames et al. (1975). The S9 was purchased from MolTox Inc. (Annapolis, MD.) and was from Aroclor 1254 pretreated male Sprague-Dawley rats. The S9 contained 40.0 mg protein/ml as determined using the method of Lowry et al (1951).

For the microsuspension assay, the following ingredients were added, in order, to a 12 x 75 mm sterile glass culture tubes kept on ice: 0.1 ml S9 mix, 0.005 ml sample in DMSO or methanol, and 0.1 ml concentrated bacteria in PBS (1 x 10^{10} / ml PBS). The mixture was incubated in the dark at 37°C with rapid shaking. After 90 min, the tubes were placed in an ice bath and taken out one at a time immediately before adding 2 ml molten top agar (Ames et al., 1975) containing 90 nmoles of histidine and biotin. The combined solutions were vortex-mixed and poured onto minimal glucose plates. Plates were incubated at 37°C in the dark for 48 hours and counted using an automatic plate counter. Strain markers were routinely determined for each experiment.

The doses tested in the bioassay were determined by taking a portion of the extract, drying the portion under a gentle stream of nitrogen, and redissolving the extract in DMSO. The highest dose was determined to be approximately 3 mg of particle equivalent per ml of DMSO. Particle equivalent refers to the amount of extract added per tube that was derived from the mass of particulate matter. For example, 3 mg particle equivalent is the amount of extract from 3 mg of particulate matter collected on the filter. The dissolved extract was then serially diluted to develop three dose levels. All doses were tested in duplicate.

The colony counts (number of revertants) represent bacteria that have mutated either spontaneously, or more typically, by exposure to genotoxic compounds. These mutant colonies were counted and analyzed by tabulating the net number of colonies (subtracting out the background or spontaneous number of colonies present from the number of colonies with mutagenic compounds). The mean number of net colonies was determined for every dose. The slope of the linear portion of the dose-reponse curve was then calculated to give a "specific mass mutagenic activity", or the number of revertants per mg particulate matter equivalent (Rev/mg). The specific mass mutagenic activity is multiplied by the mass of particulate matter emitted per mile resulting in an emission expressed as "revertants per mile". This emission factor is an indicator of genotoxic compounds released per mile.

RESULTS

The dose-response relationships of filter extracts are presented in Figures 10-13. Figures 10 and 11 represent particulate matter from the vehicle without a catalytic converter and collected during the P1 and P2 parts of the

EPA test cycle, respectively . For these emissions collected without catalyst, the specific mass mutagenic activity, or mutagenic activity per microgram mass of particulate matter, is based on the slope of the linear portion of the dose-response curve. The highest relative specific mass mutagenic activity collected during either the hot or cold test cycles was the particulate matter collected from the 100% diesel fuel emissions. The 100% diesel fuel was higher than either the 100% REE or the diesel-REE blends. For the P1 phase of the entire cycle, and without catalyst, the next highest in specific mass mutagenic activity is the 20% REE (80% diesel), followed by 50% REE (50% diesel). The lowest relative specific mass mutagenic activity was from the particulate matter collected from emissions of100% REE fuel. In general, results of the P2 part of the cycle are similar to those observed in the P1 portion of the cycle, but with a slightly higher specific mass mutagenic activity for all fuels. The order of potencies for the P2 phase without catalyst is similar to the P1 phase.

The specific mass mutagenic activity for the particulate matter extracts collected from a catalyst-equipped vehicle are illustrated in Figures 3 and 4. These tests were conducted using the P1 and P2 portion of the test cycle. The order of mutagenic potencies for P1 from highest to lowest were: 20% REE = 100% diesel > 50% REE > 100% REE. For P2, the dose-response curves appeared to be equivalent.

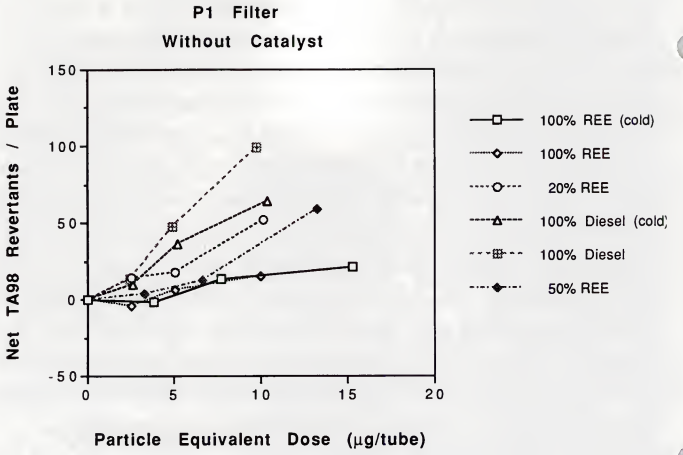


Figure 10. Dose-response curves for extracts of diesel and biodiesel particulate matter from a sample collected from the P1 portion of the EPA test cycle. Vehicle not equipped with a catalytic converter.

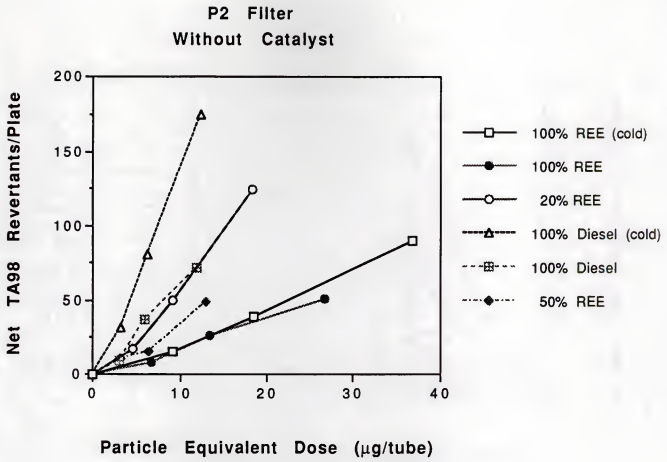


Figure 11. Dose-response curves for extracts of diesel and biodiesel particulate matter. Sample collected from the P2 portion of the EPA test cycle. Vehicle not equipped with a catalytic converter.

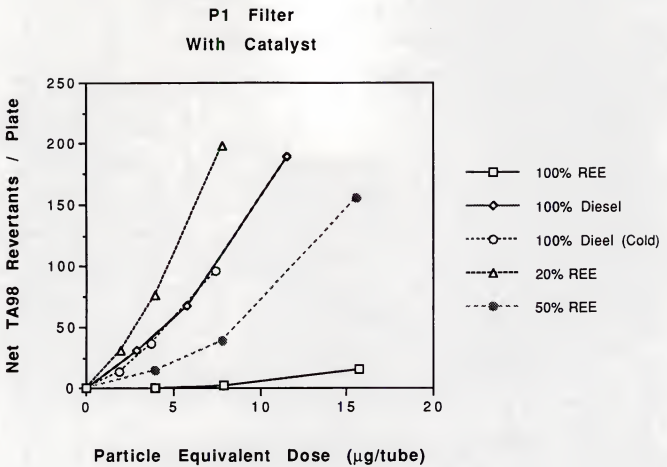


Figure 12. Dose-response curves for extracts of diesel and biodiesel particulate matter. Sample collected from the P1 portion of the EPA test cycle. Vehicle equipped with a catalytic converter.

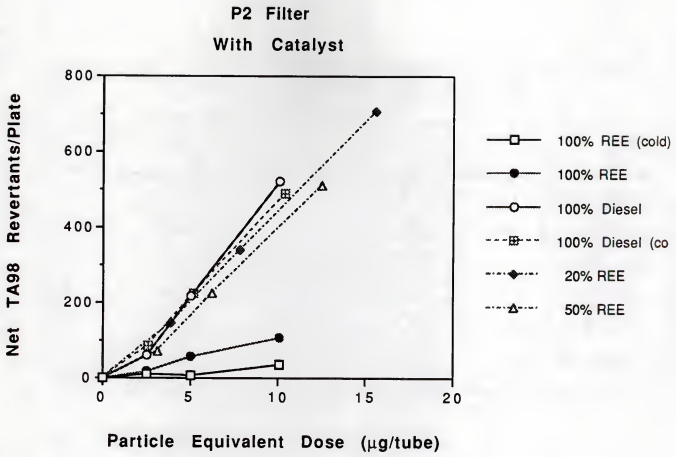


Figure 13. Dose-response curves for extracts of diesel and biodiesel particulate matter. Sample collected from the P2 portion of the EPA test cycle. Vehicle equipped with a catalytic converter.

Table 6. Specific Mass Mutagenic Activity.
Without Catalytic Converter.

Fuel	P1	P2
100% Diesel - Cold	6.43	14.39
100% Diesel - Hot	10.52	6.43
20% REE	5.02	6.99
50% REE	4.57	3.75
100% REE - Cold	1.55	2.50
100% REE - Hot	1.74	1.97

Table 7. Specific Mass Mutagenic Activity.
With Catalytic Converter.

Fuel	P1	P2
100% Diesel - Cold	13.27	48.01
100% Diesel - Hot	16.63	45.85
20% REE	25.89	53.22
50% REE	10.24	41.95
100% REE - Cold		3.18
100% REE - Hot	1.05	11.02

Emissions of Genotoxic Compounds

Emissions from the dilution tunnel were evaluated for genotoxic compounds. The emissions of genotoxic compounds from the fuels for both non-catalyst and catalyst-equipped engine are summarized in Figures 14 and 15. The emissions of mutagenic compounds are presented as "revertant equivalents per mile," where revertants are an index of genotoxic activity. The number of revertants is related to the dose and potency of the mutagenic compounds present in the extract.

Emissions of Genotoxic Compounds- Without Catalytic Converter

The emissions of genotoxic activity per mile (P1 plus P2 emissions) from the complete FTP cycle for the non-catalyst equipped engine are illustrated in Figure 14. During the complete FTP cycle, the order of emissions per mile from highest to lowest was 100% diesel (cold start), 100% diesel (hot start), 50% REE, 20% REE, 100% REE (cold start), and 100% REE (hot start). The difference from the cold start diesel to the hot start 100% REE is approximately 6.4×10^{6} revertants/ mile which is approximately 6 times the levels in the hot start REE. The hot start diesel produced approximately 4 times the emissions of the hot start100% REE.

During the P1 phase of the cycle, the exhaust from the diesel fuel in the non-catalytic engine had the highest genotoxic emissions (during both cold and hot start parts of the cycle) of the test fuels (data not shown). The cold start diesel exhaust also had the highest mutagenic activity equivalents during Phase 2. In Phase 2, the second highest emssions were from the 80% diesel (20% REE), followed by the cold start 100% REE and the hot start diesel. The

lowest activity in the P2 phase was from the hot start 100% REE and the 50% REE/diesel blend.

Emissions of Genotoxic Compounds- With Catalytic Converter

The emissions per mile (P1 and P2 emissions) from the complete FTP cycle (P1 and P2) for the catalyst-equipped engine are illustrated in Figure 15. The activity from the 100% diesel from a cold start is approximately 13.0 x 10^6 rev equiv. per mile. The next highest was the 20% REE with approximatley 9.6 x 10^6 rev equiv per mile, followed by the 100% diesel and 50% REE emissions, which had similar emissions of approximately 7 x 10^6 rev equiv per mile. The emissions for the 100% REE (hot start) were approximately 1 x 10^6 revertant equivilents per mile, or approximately 7 times lower than the hot start diesel emissions and approximately 13 times less than the cold start diesel.

The emissions for P1 and P2 phases of the FTP were investigated individually for all fuels. The highest emissions in P1 were from the diesel (cold start). The next highest were from the 20% REE (80% diesel) fuel, followed by 100% diesel (hot start) and 50% REE. The 100% REE (hot start) had the lowest emissions which were more than 20 times lower than the 100% diesel (cold start). The highest emissions during P2 were from the diesel from a cold start. The next three fuels (the hot start diesel, 20% REE and 50% REE) all had about the same mutagenic activity (approximately 5.4 x 10^6 revertant equivalents per mile.) This level of activity is approximately 14 times higher than the activity of the 100% REE from a cold start.

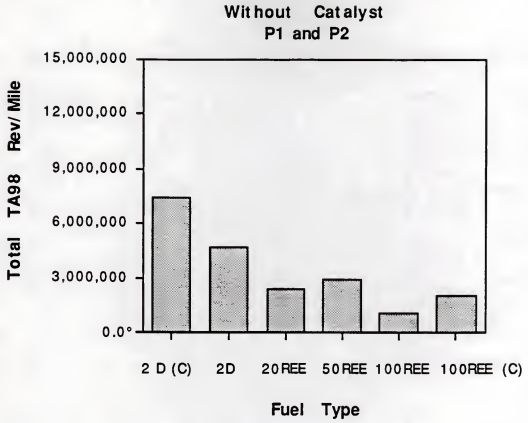


Figure 14. Total mutagenicity equivalent emissions from the diesel and biodiesel fuel. Engine not equipped with catalytic converter. All fuels were tested under hot start conditions unless indicated as a cold start (c). 2D is a100% diesel fuel.

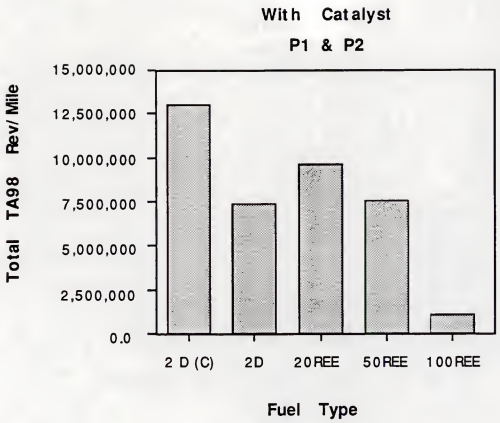


Figure 15. Total mutagenicity equivalent emissions from the diesel and biodiesel fuel. Engine equipped with catalytic converter. All fuels were tested under hot start conditions unless indicated as a cold start (c). 2D is a100% diesel fuel.

DISCUSSION

All particulate matter collected had measureable genotoxic (mutagenic) activity. All extracts of the particulate matter when tested in the Salmonella microsuspension procedure had primarily linear dose-response characteristics which is an indication that mutagenic compounds were present. The relative specific mass mutagenic activity (mutagenic activity per mass of particulate matter) provides a way to analyze relative potency of the particulate matter. This provides a description of the degreee of mutagenicity of a specific compound or complex mixture. Exposure characteristics however, depend on the emissions, or the amount of mutagenic compounds emitted per mile traveled. Since we do not know all the specific mutagenic compounds emitted, we measure mutagenic activity as an index of these compounds. The emissions therefore reported as "revertant equivalents per mile" and are dependent on the potency of the particles in combination with the mass of particles emitted. A discussion of the potency, or specific mass mutagenic activity is followed by a discussion of the emissions.

The specific mass mutagenic activity was markedly different depending on the fuel type and if the vehicle was equipped with a catalytic converter for emissions. When there was no catalytic converter, the highest relative specific mass mutagenic activity for particles collected either during the cold or hot test cycle was from the 100% diesel fuel. The specific mass mutagenic activity decreased with the increase of REE, with the 100% REE fuel having the lowest relative activity. The 100% REE activity was approximately 3 to 7 times lower than that of the 100% diesel fuel, depending on whether it was a hot or cold part of the cycle, and whether a catalytic converter was used. The REE produced significantly lower specific mass

mutagenic activity than diesel fuel when a catalyst was not used. The 100% REE and REE blends were approximately 3 times less potent per mass of particulate matter than the 100% diesel samples (Table 6).

The specific mass mutagenic activity with a catalyst was higher than the activity without a catalyst (Table 7). For the P1 part of the cycle, mutagenic activity from the 20% REE blend was higher than that of the hot or cold diesel tests. This increase was also seen in the P2 part of the cycle. The P2 part of the cycle overall had higher specific mass mutagenic activity than the P1 part of the cycle, with at least a doubling of activity for all fuels. The 100% REE fuel had approximately 10 times more in activity in the P2 than in the P1 portions of the entire test cycle, but still produced approximately 1/4 that produced by less than diesel or the blends. The nature of the P2 portion of the test cycle may have produced this increased activity with a catalyst. The high engine speeds experienced during the P2 portion of the test cycle may produce the greater amounts of mutagenic compounds. However, when no catalyst was in place, the P1 and P2 portions of the cycle appeared to be equivalent (see Table 6). The catalyst used in this study may therefore facilitate the formation of certain mutagenic compounds. The increase in the 20% REE and the similarity of all REE blends compared to the 100% diesel fuel and in the P1 and P2 portions of the test cycle with catalyst, should be further investiaged. A number of mechanisms are possible. For example, an increase in the specific mass mutagenic activity can be the result of enriching for particles that have adsorbed mutagenic compounds and eliminating possibly larger particles.

Although the activity per particle mass is important as an didicator of the the potency of the particulate matter, an important component of the analysis of human exposure is to investigate the total emissions of mutagenic

compounds, or levels of mutagenic compounds emitted per mile. The total emissions of mutagenic compounds without a catalytic converter followed the rank order of specific mass mutagenic acivity: 100% diesel cold start) > 100% diesel (hot start) > 50% diesel > 20% REE > 100 % REE (cold start) > 100% REE (hot start). The emissions per mile from the hot start 100% REE fuel without a catalytic converter are approximately 4 times lower than the 100% diesel fuel.

The emissions from the catalytst-equipped truck had a rank order pattern similar to those of its specific mass mutagenic activity. The 100% diesel fuel (cold start) had the highest emissions and this was followed by the 20% REE (80% diesel). The hot start 100% diesel and 50% REE were approximately equivalent in emissions, but slightly lower than the 20% REE fuel. The 100% REE had the lowest emissions as was the case when there was no catalyst. The use of catalyst with fuel blended with REE results in a small reduction in the emissions of mutagenic compounds from those found in the 100% diesel fuel. These results suggests that the catalyst is not functioning in the manner intended for diesel particulate matter. The catalyst with the fuels tested here needs further investigation.

These studies would benefit from a replication using larger sample size, and a trapping of the vapor-phase compounds in conjunction with the trapping and analyses of the particulate matter. The vapor-phase mutagenic compounds could then be compared to the particle phase and a more complete profile of emissions could be obtained. Further, the emissions with and without a catalyst need further investigation measuring both particle and vapor-phase. Finally, two procedural approaches are recommended for incorporation into the test plan: 1) tunnel blanks where a sampling of the tunnel without the engine running and conducted for identical times as the

test cycle is recommended. 2) tunnel conditioning where filtered ambient air is drawn through the system for specified times prior to testing the next fuel is recommended to be incorporated into the test plan.

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Appendix 1

QUALITY ASSURANCE

Quality assurance (QA) procedures were incorporated into the experimental design to include reagent blanks, reagent spikes, filter blanks, duplicate samples and control samples. In addition, performance standards were specified to evaluate the quality of the data. A summary of QA samples, procedures and standards are summarized.

Reagent blank: A reagent blank undergoes all the same preparative and analytical steps as the sample to be analyzed. A reagent blank should be incorporated with each set of samples. In general, levels of target compounds in the reagent blank should be less than 20% of the levels measured in the sample.

Reagent spike: A reagent spike is a sample spiked with a known amount of target analytes. The reagent spike also undergoes all the same preparative and analytical steps as the sample. Recoveries of the target analytes spiked in the reagent blank should be between 65 and 135% of the known amount.

Filter blanks: A blank filter is not used in sampling, but is ideally from an identical lot of the sample filter. The filter blank is analyzed for background levels of PAHs. Levels of any target analyte in the filter blank should not exceed 20% of the levels in the actual sample.

Duplicate Analysis: Duplicate analysis is performed to estimate the precision of the analysis. Each analysis should be within ±20% of the average. A minimum of 10% of the samples are run in duplicate.

Calibration Check Samples: A calibration check sample is a standard of known concentration that is run for each set of samples after every 10th experimental sample. The calibration check is performed to determine if there is a change in instrument (GC/MS) response during the analysis of a set of samples. Variation from the initial calibration should not exceed +/-20%. Calibration Check sample results are summarized in Table A1-1. For all PAHs measured, no values exceeded 20% of the initial calibration.

Table A1-1 Calibration Check Results.

Compound	Check Sample 1 Conc. (pg/µl)	% Bias*	Check Sample 2 Conc. (pg/µl)	% Bias*
Naphthalene	85.0	6.2	80.7	0.825
Acenaphthylene	94.0	17.5	73.5	-8.11
Acenaphthene	83.1	3.85	74.8	-6.45
Fluorene	78.5	-1.83	81.5	1.88
Phenanthrene	78.3	-2.16	69.6	-13.1
Anthracene	95.7	19.6	89.1	11.4
Flouranthene	84.2	5.28	85.4	6.78
Pyrene	86.4	8	82.9	6.78
Benzo[a]anthracene	96.3	20.3	82.5	3.63
Chrysene	86.3	7.9	86.1	3.15
Benzo[b]Flouranthene	77.0	-3.79	82.02	7.59
Benzo[k]flouranthene	85.7	7.08	86.5	2.56
Benzo[e]pyrene	66.8	-16.6	77.5	8.06
Benzo[a]pyrene	71.5	-10.7	75.4	-3.16
Perylene	74.4	-6.98	76.4	-5.71
Indeno[1,2,3-cd]pyrene	76.2	-4.73	78.8	-4.45
Dibenz[ah]anthracene	83.0	3.74	82.8	-1.49
Benzo[ghi]perylene	79.4	-0.763	82.5	3.46

% Bias = percent difference from the known value.

Compound identification based on GC retention time and MS target ions:

The qualifier ion ratios should be within 20% of the ratios of authentic standards. Background subtraction is used to meet this criterion. For analytes near the RQL this criterion often could not be met and compound identifications were based only on the target ion and the retention time.

The reagent spike results are listed in Table A1-2. One reagent spike was run at 200 pg/µl. A second reagent spike was run at 100 pg/µl. One spike sample extract evaporated to dryness due to a faulty cap. This sample was reconstituted with the addition of 300 ul of DCM before analysis. The high recovery value for naphthalene (*) and the low recovery for acenaphthene (*) were probably due to evaporative loss of DCM prior to analysis.

Table A1-2. Reagent Spike Results.

Compound	Spike 1 (200 pg/ul) Conc. (pg/µl)	%Recovery	Spike 2 (100 pg/µl) Conc. (pg/µl)	%Recovery
Naphthalene	218.	109.	812	813*
Acenaphthylene	180.	90.0	107	107
Acenaphthene	200	100	107	107
Fluorene	157	78.5	24.9	24.9*
Phenanthrene	191	95.7	110	110
Anthracene	119	59.3*	84.3	84.3
Flouranthene	190	95.2	106	106
Pyrene	191	95.6	113	113
Benzo[a]anthracene	171	85.7	91.0	91.0
Chrysene	189	94.6	96.8	96.8
Benzo[b]Flouranthene	189	94.51	88.7	88.7
Benzo[k]flouranthene	198	98.8	95.2	95.2
Benzo[e]pyrene	**	**	**	**
Benzo[a]pyrene	146.	73.2	86.9	86.9
Perylene	**	**	**	**
Indeno[1,2,3-cd]pyrene	196	98	90.8	90.8
Dibenz[ah]anthracene	192	96	92.7	92.7
Benzo[ghi]perylene	182	91.0	95.9	95.9

* Values outside acceptable recoveries.
** = These PAHs were not in the reagent spike

Duplicate Analysis

The results of the duplicate analysis are summarized in Table A1-3. Five sets of samples were each run in duplicate, exceeding the 10 percent duplicate analysis requirement. Some percent differences could not be calculated because one value was either above or below the RQL. For sample

set 2, only the reported value for anthracene was outside the ±20 % difference. For some compounds in each sample set, duplicate analysis could not be calculated because both values were below the RQL.

Table A1-3. Duplicate Analyses of Samples and Percent Differences.

Compound	Sample ID Set 1			Sample ID Set 2			Sample ID Set 3		
	1451	1451	% diff	1454	1454	% diff	1456	1456	% diff
Naphthalene	27.6	30.8	-5.5	40.8	41.9	-1.3	43	39.8	3.9
Acenaphthylene		9.5	*		29.4	*	BDL	BDL	
Acenaphthene	BDL	BDL		BDL	BDL		BDL	BDL	
Fluorene	BDL	BDL		33.2		*	BDL	BDL	
Phenanthrene	209	207	0.5	743	700	3.0	202	202	0.0
Anthracene	BDL	BDL		63.5	36.6	26.9 #	BDL	BDL	
Fluoranthene	99.3	119	-9.0	246	268	-4.3	79	81.8	-1.7
Pyrene	228	211	3.9	556	572	-1.4	201	226	-5.9
Benz[a]anthracene	23.4	24	-1.3	59.7	53.6	5.4	20.1	15.6	12.6
Chrysene/Triphenylene	29.4	33.5	-6.5	82.4	78.9	2.2	28	26.1	3.5
Benzo[b]fluoranthene	19.3	21.2	-4.7	44.8	50.8	-6.3	13.4	15.4	-6.9
Benzo[k]fluoranthene	BDL	BDL		12	15.4	-12.4	BDL	BDL	
Benzo[e]pyrene	10.4	11.9	-6.7	22.5	25.5	-6.3	9	10.4	-7.2
Benzo[a]pyrene	BDL	BDL		19	18.9	0.3	BDL	BDL	
Perylene	9.5		*	10.9		*	BDL	BDL	
Indeno[1,2,3-cd]pyrene	BDL	BDL		21.1	22.6	-3.4	BDL	BDL	
Dibenz[ah]anthracene	BDL	BDL		BDL	BDL	BDL	BDL	BDL	
benzo[ghi]perylene	12.3	13.4	-4.3	29	29.1	-0.2	10.8	10	3.8

Compound	Sample ID Set 4			Sample ID Set 5		
	1437	1437	% diff	1443	1443	% diff
Naphthalene	49	50.8	-1.8	47.1	44.3	3.1
Acenaphthylene	BDL	BDL		BDL	BDL	
Acenaphthene	BDL	BDL		BDL	BDL	
Fluorene	BDL	BDL		BDL	BDL	
Phenanthrene	548	532	1.5	96.4	94.7	0.9
Anthracene	37	37.8	-1.1	BDL	BDL	
Fluoranthene	171	178	-2.0	90.4	91.1	-0.4
Pyrene	270	243	5.3	116.4	116.9	-0.2
Benz[a]anthracene	30.7	26.2	7.9	BDL	BDL	
Chrysene/Triphenylene	42.3	47.5	-5.8	29.5	27.8	3.0
Benzo[b]fluoranthene	24.2	27.7	-6.7	12.1	12.7	-2.4
Benzo[k]fluoranthene	BDL	BDL		BDL	BDL	
Benzo[e]pyrene	12.8	15.7	-10.2	6.6	8.1	-10.2
Benzo[a]pyrene	14	16.7	-8.8	BDL	BDL	
Perylene	BDL	BDL		BDL	BDL	
Indeno[1,2,3-cd]pyrene	15.9	17.4	-4.5	BDL	BDL	
Dibenz[ah]anthracene	BDL	BDL		BDL	BDL	
benzo[ghi]perylene	21.3	24.7	-7.4	BDL	BDL	

* Percent different could not be calculated because one value was below the quantification limit
# Percent different greater than +/- 20%
BDL = Below Detection Limit

Blank Results

One filter blank and two reagent blanks were analyzed. The blank results are summarized in Table A1-4.

Table A1-4. Filter and Reagent Blank Results.

Compound	Filter Blank (pg/µl)	Reagent Blank 1[a] (pg/µl)	Reagent Blank 2[b]
Naphthalene	57.0	49.2	ND
Acenaphthylene	<12.7	<12.7	ND
Acenaphthene	<8.58	<8.58	ND
Flourene	<15.2	<15.3*	ND
Phenanthrene	11.8	11.8	ND
Anthracene	<24.8	<24.8	ND
Flouranthene	<4.85*	44.6	ND
Pyrene	<3.45*	252	ND
Benzo[a]anthracene	<16	37.5	ND
Chrysene	<9.91	22.9	ND
Benzo[b]Flouranthene	<10.9	<10.9	ND
Benzo[k]flouranthene	<10.9	<10.9	ND
Benzo[e]pyrene	<2.76*	<2.76*	ND
Benzo[a]pyrene	<9.0*	<9	ND
Perylene	<8.1	14.1	ND
Indeno[1,2,3-cd]pyrene	<16	16	ND
Dibenz[ah]anthracene	<8.03	<8.03*	ND
Benzo[ghi]perylene	<9.31	<9.31*	ND

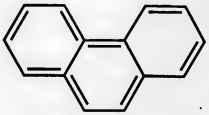
[a] Reagent blank contaminated by autosampler that was not processed through a cleaning cycle.
[b] ND = not detected

The filter blank contained 57 pg/µl of naphthalene. Naphthalene in the filter blank was at comparable levels to those in the samples and exceeded the 20% blank level performance standard. Phenanthrene in the filter blank

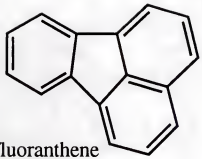
was 11.8 pg/µl, which was far less than 20 % of the phenanthrene detected in any of the blank samples. Benzo[a]pyrene (BaP) and benzo[e]pyrene (BeP) were detected in the filter blank, but the amounts were well below the RQL. BaP in samples 1436, 1440, 1443, 1451, and 1456 is also below the RQL. The amount of BaP in the blank filter was less than 20% of the value in the samples reported. BeP was detected in samples 1436, 1440 and 1443, but was below the RQL and may be at comparable concentrations to those found in the blank filter. The amount of BeP in the blank was less than 20% of the values reported in the samples. Flouranthene and pyrene were detected but were below the RQL in the blank filter and were far below levels detected in any of the samples. No other PAHs were detected in the filter blank.

An internal standard was not added to the first reagent blank and quantitative results were not obtained. No PAHs were detected in this reagent blank. The second reagent blank contained high levels of pyrene and fluoranthene, probably caused by an improper cleaning cycle of the autosamper syringe. The reagent blank was the first sample in the batch of samples analyzed. The autosampler cleans the syringe after each analysis and no carry over was observed in subsequent calibration runs. The results did not affect any of the samples that were analyzed.

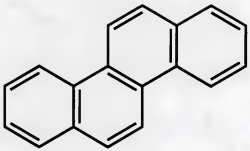
Appendix 2. Representative PAH structures



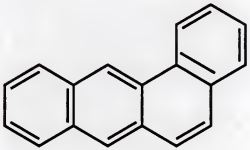
Phenanthrene



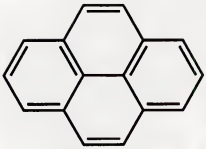
Fluoranthene



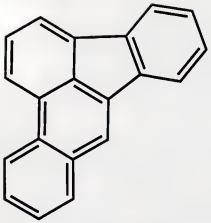
Chrysene/Triphenylene



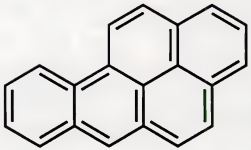
Benz [a] anthracene



Pyrene



Benzo [b] fluoranthene



Benzo [a] pyrene

Benzo [ghi] perylene

Appendix 3. Concentrations of PAHs on Filter Samples.

TABLE A3-1. TOTAL PAHS PER POOLED SAMPLE (P1+P2) FROM A CATALYST-EQUIPPED DIESEL VEHICLE.

Sample ID	1430	1437	1433	1443	1440	1436
Filter ID	P1+P2	P1+P2	P1+P2	P1+P2	P1+P2	P1+P2
Percent Diesel	0%	100%	0%	50%	80%	100%
Catalyst	Yes	Yes	Yes	Yes	Yes	Yes
Hot/Cold Start	Cold	Cold	Hot	Hot	Hot	Hot
Filter Factor	0.4914	0.4938	0.5097	0.5066	0.4975	0.4988
			TOTAL PAH MASS (ng)			
COMPOUND						
Naphthalene	40.59	30.30	180	73.76	24.69	83.19
Acenaphthylene	19.73	<3.81	17.74	<8.26	<3.81	<8.26
Acenaphthene	20.35	<2.57	19.86	<5.58	<2.57	<5.58
Fluorene	10.33	<3.66	6.23	<7.98	<3.66	<7.98
Phenanthrene	65.23	328	78.74	167	112	114
Anthracene	25.31	22.71	21.07	<16.12	<7.44	<16.1
Fluoranthene	113	106	107	105	81.29	81.60
Pyrene	144	178	104	137	126	123
Benz[a]anthracene	33.55	17.29	25.51	<10.4	<4.80	<10.4
Chrysene/Triphenylene	40.83	27.29	31.95	16.96	22.63	<6.44
Benzo[b]fluoranthene	40.65	15.77	25.59	7.35	8.38	<7.09
Benzo[k]fluoranthene	30.50	<3.27	22.85	<7.09	<3.27	<7.09
Benzo[e]pyrene	13.02	8.65	5.96	4.36	<0.83	<1.79
Benzo[a]pyrene	36.06	9.33	21.12	<5.85	<2.70	<5.85
Perylene	<2.43	<2.43	5.27	<5.27	<2.43	<5.27
Indeno[1,2,3-dc]pyrene	39.58	10.13	21.80	<10.4	<4.80	<10.4
Dibenz[ah]anthracene	25.63	<2.41	21.88	<5.22	<2.41	<5.22
Benzo[ghi]perylene	54.04	13.97	25.17	<6.05	<2.79	<6.05

Ng PAH = total nanograms of PAH per pooled sample

< = less than the method detection level

TABLE A3-1. TOTAL PAHS PER POOLED SAMPLE (P1+P2) FROM A DIESEL VEHICLE WITHOUT CATALYST (CONT).

Sample ID	1445	1454	1448	1458	1451	1456
Filter ID	P1,P2	P1,P2	P1,P2	P1,P2	P1,P2	P1,P2
Percent Diesel	0%	100%	0%	50%	80%	100%
Catalyst	No	No	No	No	No	No
Hot/Cold Start	Cold	Cold	Hot	Hot	Hot	Hot
Filter Factor	0.4929	0.4805	0.512	0.5	0.5062	0.4934
			TOTAL PAH MASS (ng)			
COMPOUND						
Naphthalene	27.58	25.82	26.35	31.55	17.31	25.19
Acenaphthylene	<3.81	<3.81	<3.81	<3.81	<3.81	<3.81
Acenaphthene	<2.57	<2.57	<2.57	<2.57	<2.57	<2.57
Fluorene	<3.66	<3.66	<3.66	<3.66	<3.66	<3.66
Phenanthrene	330	451	122	132	123	123
Anthracene	<4.11	31.25	<4.11	<4.11	<4.11	<4.11
Fluoranthene	348	160	72.53	62.39	64.62	48.88
Pyrene	203	352	111	111	130	130
Benz[a]anthracene	27.76	35.37	11.17	13.68	14.03	10.87
Chrysene/Triphenylene	30.82	50.36	13.96	17.68	18.63	16.43
Benzo[b]fluoranthene	32.79	29.84	13.13	15.77	12.01	8.75
Benzo[k]fluoranthene	25.11	8.54	<3.27	<3.27	<3.27	<3.27
Benzo[e]pyrene	16.69	15.00	7.69	8.63	6.60	5.88
Benzo[a]pyrene	16.58	11.83	6.56	6.17	<.83	<.83
Perylene	8.41		5.42	5.04	<2.70	<2.70
Indeno[1,2,3-dc]pyrene	19.31	13.66	<4.80	<4.80	<4.80	<4.80
Dibenz[ah]anthracene	<2.41	<2.41	<2.41	<2.41	<2.41	<2.41
Benzo[ghi]perylene	34.19	18.14	10.85	10.85	7.63	6.31

Ng PAH = Nanograms of PAH per pooled sample

< = less than the method detection level

TABLE A3-2. CONCENTRATION OF PAHs FROM A CATALYST-EQUIPPED DIESEL VEHICLE.

Sample ID	1430	1437	1433	1443	1440	1436
Filter ID	P1P2	P1,P2	P2	P2	P1,P2	P1
Percent Diesel	0%	100%	0%	50%	80%	100%
Catalyst	Yes	Yes	Yes	Yes	Yes	Yes
Hot/Cold Start	Cold	Cold	Hot	Hot	Hot	Hot
Filter mass (mg)	2.066	2.798	1.408	1.727	1.574	1.261
	PAH CONCENTRATION (ng PAH/mg particulate matter)					
COMPOUND						
Naphthalene	19.65	10.83	128	42.71	15.69	65.94
Acenaphthylene	9.55	<1.36	12.60	<4.78	<2.42	<6.55
Acenaphthene	9.85	<.918	14.11	<3.23	<1.63	<4.42
Fluorene	5.00	<1.31	4.43	<4.62	<2.33	<6.33
Phenanthrene	31.57	117.28	55.92	96.46	70.88	90.55
Anthracene	12.25	8.12	14.96	<9.33	<4.73	<12.8
Fluoranthene	54.88	37.91	76.30	60.76	51.64	64.68
Pyrene	69.47	63.54	74.16	79.44	80.03	97.67
Benz[a]anthracene	16.24	6.18	18.12	<6.02	<3.05	<8.24
Chrysene/Triphenylene	19.76	9.75	22.69	9.82	14.37	<5.11
Benzo[b]fluoranthene	19.67	5.64	18.17	4.25	5.32	<5.62
Benzo[k]fluoranthene	14.76	<1.17	16.23	<4.11	<2.08	<5.62
Benzo[e]pyrene	6.30	3.09	4.23	2.52	<.527	<1.42
Benzo[a]pyrene	17.45	3.33	15.00	<3.39	<1.72	<4.64
Perylene	<1.18	<.868	<3.74	<3.05	<1.54	<4.18
Indeno[1,2,3-dc]pyrene	19.16	3.62	15.48	<6.02	<3.05	<8.24
Dibenz[ah]anthracene	12.41	<1.00	15.54	<3.02	<1.53	<4.14
Benzo[ghi]perylene	26.15	4.99	17.87	<3.50	<1.77	<4.80

Ng/mg = Nanograms of PAH per milligram of particulate matter

< = less than the method detection level

TABLE A3-2. CONCENTRATION OF PAHs FROM VEHICLE NOT EQUIPPED WITH CATALYST (CONT).

Sample ID	1445	1454	1448	1458	1451	1456
Filter ID	P1,P2	P1,P2	P1,P2	P1,P2	P1,P2	P1,P2
Percent Diesel	0%	100%	0%	50%	80 %	100%
Catalyst	No	No	No	No	No	No
Hot/Cold Start	Cold	Cold	Hot	Hot	Hot	Hot
Filter mass (mg)	5.678	4.228	3.108	2.956	2.301	2.215
	PAH CONCENTRATION (ng PAH/mg particulate matter)					
COMPOUND						
Naphthalene	4.86	6.11	8.48	10.67	7.52	11.37
Acenaphthylene	<.671	<.901	<1.22	<1.29	<1.66	<1.72
Acenaphthene	<.453	<.608	<.827	<.869	<1.12	<1.16
Fluorene	<.645	<.866	<1.18	<1.24	<1.59	<1.65
Phenanthrene	58.17	107	39.30	44.74	53.54	55.52
Anthracene	<1.31	7.39	<2.39	<2.52	<3.23	<3.36
Fluoranthene	61.21	37.93	23.34	21.11	28.08	22.07
Pyrene	35.78	83.30	35.61	37.39	56.48	58.63
Benz[a]anthracene	4.89	8.36	3.60	4.63	6.10	4.91
Chrysene/Triphenylene	5.43	11.91	4.49	5.98	8.10	7.42
Benzo[b]fluoranthene	5.78	7.06	4.22	5.33	5.22	3.95
Benzo[k]fluoranthene	4.42	2.02	<1.05	<1.11	<1.42	<1.48
Benzo[e]pyrene	2.94	3.55	2.48	2.92	2.87	2.65
Benzo[a]pyrene	2.92	2.80	2.11	2.09	<1.17	<1.22
Perylene	1.48	<.575	1.74	1.71	<1.06	<1.10
Indeno[1,2,3-dc]pyrene	3.40	3.23	<1.54	<1.62	<2.09	<2.17
Dibenz[ah]anthracene	<.424	<.570	<.775	<.815	<1.05	<1.09
Benzo[ghi]perylene	6.02	4.29	3.49	3.67	3.31	2.85

ng/mg = nanograms of PAH per milligram of particulate matter

< = less than the method detection level